coupling of agonist binding to gating of the ion channel, indicating that agonist binding and subsequent ion channel opening are separate, but related processes. Phy differentially displaces [^{125}I]α-BGT from the chimeric nAChR, suggesting that the β subunit is not involved in Phy binding, and that Phy targets the insect agonist binding loop C.

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Imidazenil, a new drug for the management of convulsions in organophosphate intoxication in rodents

Slawomir Rump,* Teresa Gidynska, Elzbieta Galecka, Oktawiusz Antkowiak and Marek Kowalczyk

Department of Pharmacology and Toxicology, Military Institute of Hygene and Epidemiology, 4 Kozielska Str, 01-163 Warsaw, Poland

* Correspondence to: Slawomir Rump, Department of Pharmacology and Toxicology, Military Institute of Hygene and Epidemiology, 4 Kozielska Str, 01-163 Warsaw, Poland

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Abstract: The summary deals with the anti-convulsant and antilethal effects of a new benzodiazepine receptor partial agonist, imidazenil, in DFP intoxication. It has been demonstrated that imidazenil (2–5 mg kg^{-1}) significantly decreases convulsion intensity, rapidly inhibits seizure patterns in brain bioelectrical activity and significantly increases the anti-lethal efficacy of atropine plus obidoxime therapy. These effects are comparable to diazepam at 5 mg kg^{-1}. However, diazepam exhibits myorelaxant activity at therapeutic doses, which are only observed at 5–10 times the therapeutic doses of imidazenil.

Keywords: benzodiazepines; convulsions; DFP; imidazenil; organophosphate

1 INTRODUCTION

Centrally mediated seizures are one of the toxic signs following poisoning with organophosphates (OP), Benzodiazepines (BDZ), especially diazepam, are very effective in the management of these convulsions when given as adjuncts to atropine and oxime.[1,2] However, sedation, myorelaxation and dependence make BDZ a poor choice for the treatment in these states. Partial BDZ receptor agonists are regarded as producing these side effects only in very high doses. Some of these drugs, like compound CGS9896, are very effective in the treatment of OP intoxications[3] but unfortunately they are not registered as drugs. The recently reported partial allosteric modulator of BDZ receptors, imidazenil (6-(2-bromophenyl)-8-fluoro-4*H*-imidazo [1,5-*a*] benzodiazepine-3-carboximide),[4] is more like the ideal drug for the management of OP-induced convulsions. This drug is now under extensive studies in many laboratories and clinics and is considered as a potential novel anti-epileptic drug.[5] The present study was performed in order to determine anti-convulsant effects of imidazenil in acute OP intoxications, as well as to establish its antidotal efficacy in rodents.

2 MATERIAL AND METHODS

2.1 Tested drugs

Fluostigmine (diisopropyl phosphorofluoridate; DFP) was used as a model OP compound

2.2 Anti-convulsant efficacy

2.2.1 Effects on convulsion intensity

These were determined on 20 Swiss strain male mice divided in three groups (control and two experimental) using a Convulsometer (Columbus Instruments, USA). The control group received DFP (5 mg kg^{-1} sc) and obidoxime (40 mg kg^{-1} ip) and experimental groups additionally imidazenil (2 mg kg^{-1} ip) or diazepam (5 mg kg^{-1}) immediately after the intoxication. Intensity of subsequent convulsions was measured 10, 30, 60 and 120 min after the treatment and presented in g s^{-1}

2.2.2 Effect on seizure bioelectrical activity of the brain
This was studied on 12 Wistar strain male rats divided into two groups (control and experimental). Bioelectrical activity was registered every 10 min for 60 min in the control group receiving DFP (8 mg kg^{-1} sc) and obidoxime (40 mg kg^{-1} ip) and in the experimental group treated additionally with imidazenil (5 mg kg^{-1} ip). Four stages of intensity of seizure activity were determined according to Lallement *et al*:[6] stage 1 – absence of spikes or sharp waves; stage 2 – discrete spikes and sharp waves on a normal background; stage 3 – high voltage spikes and sharp waves on a suppressed background and stage 4 – continous or bursting high voltage spiking.

2.3 Antidotal efficacy
Antidotal efficacy was examined on 96 Swiss strain male mice as the influence of imidazenil (2 mg kg^{-1} ip) on the change of 24-h LD_{50} value of DFP given sc compared to the effectiveness of the standard therapy consisted of atropine (10 mg kg^{-1} ip) and obidoxime (40 mg kg^{-1} ip), using Thompson's method.[7] Four groups are included to calculate the LD_{50} value and six animals were used in each group.

2.4 Effect on motor coordination
The effect on motor coordination was studied on 40 Swiss strain male mice using a Rota-rod Treadmill (Ugo Basile, Italy) according to the technique described by Kuribara *et al.*[8] Results were expressed as time to remain on the rod according to the 7-stage score (0: 0–4 s; 1st: 5–9 s; 2nd: 10–14 s; 3rd: 15–19 s; 4th: 20–24 s; 5th: 25–29 s; 6th: >30 s).

3. RESULTS
3.1 Anti-convulsant efficacy
3.1.1 Effect on convulsion intensity
Imidazenil in a dose of 2 mg kg^{-1} had decreased the convulsions intensity in 10 min from 38.9 (±6.1) to 7.7 (±1.6) g s^{-1}. These effects persisted. Intensity of convulsions in control groups was 16.7 (±1.9) after 60 min and 14.5 (±3.2) g s^{-1} after 120 min, whereas after imidazenil administration the values were 4.6 (±1.1) and 2.6 (±0.9) g s^{-1}, respectively. Diazepam in a dose of 5 mg kg^{-1} had decreased the convulsions intensity in 10 min to 7.3 g s^{-1}.

That effect also persisted, and after 60 min the intensity of convulsions following the treatment with diazepam was 2.12 (±0.6) g s^{-1} and after 120 min 1.31 (±0.5) g s^{-1}.

3.1.2 Effects on seizure bioelectrical activity of the brain
Administration of DFP resulted in seizure electrical activity corresponding to stage 4 which slowly diminished to the mean value of 3.2 in 60 min. Administration of imidazenil resulted in a decrease of intensity to the mean value of 3.5 and in 40 min down to 2.0. Complete normalization of the record was not observed during the observation period.

Table 1. Effects of imidazenil given as adjunct to the standard therapy on toxicity of DFP in the mouse

Treatment (mg kg^{-1} ip)	*LD_{50} (mg kg^{-1} sc) (95% CL)*[a]	*TI*[b]
DFP	5.45 (4.99–5.95)	–
+ atropine (10) + obidoxime (40)	215.1 (159.5–290.1)	39.5
+ atropine (10) + obidoxime (40) + imidazenil (2)	400.0 (329.6–485.3)	73.4
+ atropine (10) + obidoxime (40) + diazepam (5)	348.9 (283.6–429.3)	64.0

[a] 24 animals per group.
[b] TI = Therapeutic Index (LD_{50} with treatment: LD_{50} without treatment).

3.2 Antidotal efficacy
This is shown in Table 1. Effect of imidazenil (2 mg kg^{-1}) was not significantly different from that of diazepam (5 mg kg^{-1}).

3.3 Effect on motor coordination
Imidazenil in doses higher than 10 mg kg^{-1} significantly decreased the motor performance (mean score 1.5) whereas diazepam elicited strong myorelaxant activity at 5 mg kg^{-1} (score 0.6). To achieve the same level of myorelaxant activity as diazepam at 5 mg kg^{-1} a dose of imidazenil at 25 mg kg^{-1} was needed (mean score 0.6 and 0.5, respectively)

4 DISCUSSION
Diazepam is now commonly used for the management of convulsions in OP intoxications. The results presented here indicated that the effectiveness of imidazenil at 2 mg kg^{-1} in the management of DFP-induced convulsions is very close to that of diazepam at 5 mg kg^{-1}. The antidotal effects were very similar. However, diazepam in therapeutic doses (5 mg kg^{-1} in the mouse) produces very strong disturbance in motor coordination. Such an effect was observed after administration of imidazenil in a dose of 25 mg kg^{-1}, ie >10 times higher than therapeutic dose (2 mg kg^{-1} in the mouse), which is of great practical advantage. Registration of imidazenil as a licenced drug could made this compound a drug of choice for the management of convulsions in human OP intoxications.

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The action of pyrethroids on the voltage-sensitive calcium channel of *Paramecium tetraurelia*

Steven B Symington,* Aiguo Zhang and J Marshall Clark
Environmental Science Program, University of Massachusetts, Amherst, MA 01003, USA

Abstract: Calcium regulation is an important event in synaptic transmission and neuronal function, which is governed by a very intricate signal transduction system which is not completely understood. Using a variety of pharmacological assays, we have characterized the action of deltamethrin on the ciliary voltage-sensitive calcium channel and on phospholipase C activity of *Paramecium tetraurelia* Sonneborn, an organism that does not possess a voltage-sensitive sodium channel. In fura-2 fluorometric assays, which examined whole cells and ciliary membrane vesicles enriched with calcium channels, deltamethrin stimulated Ca^{2+} uptake. We also determined that the phospholipase C activity of the ciliary membrane vesicles is regulated by the βγ-subunit from heterotrimeric G-proteins. Subsequent treatment with deltamethrin resulted in a substantial and highly significant increase in phospholipase C activity. These results provide evidence that the molecular mode of action of pyrethroids on the voltage-sensitive calcium channel is distinct from the action of this insecticide on the voltage-sensitive sodium channel and may be dependent, in part, upon an interaction with the βγ-subunit of heterotrimeric G-protein.

Keywords: pyrethroids; voltage-sensitive calcium channel; G-proteins; phospholipase C; *Paramecium tetraurelia*

* Correspondence to: Steven B Symington, Environmental Science Program, University of Massachusetts, Amherst, MA 01003, USA
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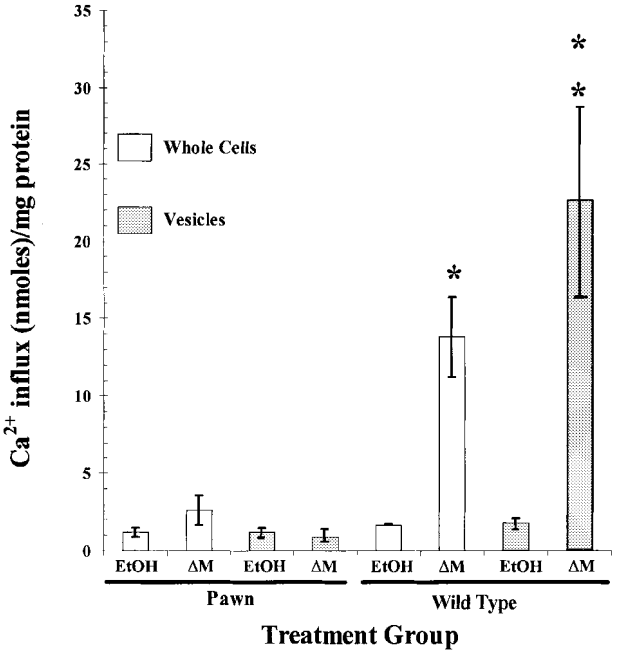

Figure 1. Fluorescent determination of the effect of 10^{-7}M deltamethrin (ΔM) on Ca^{2+} influx in whole cells and ciliary calcium-channel-containing membrane vesicles from *Paramecium tetraurelia*. (*) indicates that deltamethrin treatment is significantly different from ethanol control ($p < 0.05$). (**) indicates that deltamethrin treatment is significantly different from ethanol control ($p < 0.06$).

Type II pyrethroids, including deltamethrin, are toxic to *Paramecium tetraurelia* Sonneborn, an organism that does not possess a voltage-sensitive sodium channel. In behavioral bioassays, deltamethrin-treated cells exhibited an increase in backward swimming, a well-characterized avoidance response controlled by the voltage-sensitive calcium channel. The non-toxic 1*S* isomer of deltamethrin had no significant effect on either mortality or avoidance behavior of *Paramecium*. *Pawn* mutants, which lack a functional voltage-sensitive calcium channel, were likewise unaffected by deltamethrin. Intracellular recordings of whole cells showed that 10^{-9}M deltamethrin resulted in membrane destabilization, increased spontaneous action potentials, repetitive discharges, and membrane depolarization. Our initial findings established that the toxic effect of deltamethrin is structurally related, dose-dependent, and enhanced by depolarization, thus providing evidence that type II pyrethroids, specifically deltamethrin, act as potent calcium channel agonists in *P tetraurelia*.[1]

Figure 1 compares the effect of deltamethrin on Ca^{2+} influx in whole cells vs enriched calcium-channel-containing membrane vesicles as measured by fura-2 fluorometric assays. As expected, deltamethrin treatment of *pawn* mutants resulted in no significant increase in internal free [Ca^{2+}] in either whole cell or membrane vesicle assays. In similar experiments with wild-type cells, deltamethrin treatment increased internal free [Ca^{2+}] 8-fold in whole cells and 12-fold in the membrane vesicle preparations. These findings further substantiate our initial